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Antiviral activity of the dichloromethane extracts from *Artocarpus heterophyllus* leaves against hepatitis C virus

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ABSTRACT

Objective: To determine anti-viral activities of three *Artocarpus* species: *Artocarpus altilis*, *Artocarpus camansi*, and *Artocarpus heterophyllus* (*A. heterophyllus*) against Hepatitis C Virus (HCV).

Methods: Antiviral activities of the crude extracts were examined by cell culture method using Huh7it-1 cells and HCV genotype 2a strain JFH1. The mode of action for anti-HCV activities was determined by time-of-addition experiments. The effect on HCV RNA replication and HCV accumulation in cells were analyzed by quantitative reverse transcription-PCR and western blotting, respectively.

Results: The dichloromethane (DCM) extract of *A. heterophyllus* exhibited strong anti-HCV activity with an inhibitory concentration (IC_{50}) value of (1.5 ± 0.6) μg/mL without obvious toxicity. The DCM extracts from *Artocarpus altilis* and *Artocarpus camansi* showed moderate anti-HCV activities with IC_{50} values being (6.5 ± 0.3) μg/mL and (9.7 ± 1.1) μg/mL, respectively. A time-of-addition studies showed that DCM extract from *A. heterophyllus* inhibited viral entry process though a direct virucidal activity and targeting host cells. HCV RNA replication and HCV protein expression were slightly reduced by the DCM treatment at high concentration.

Conclusions: The DCM extract from *A. heterophyllus* is a good candidate to develop an antiviral agent to prevent HCV grant reinfection following liver transplantation.

1. Introduction

Hepatitis C Virus (HCV) infection is a major health problems that lead to liver diseases such as chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Approximately 170 million people of world population are chronically infected with HCV [1–3]. HCV exhibits high genetic diversity and different genotypes which are classified into seven (1–7) genotypes with 67 confirmed and 20 provisional subtypes [4]. In

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HCV-positive patients, the cumulative risk of developing hepatocellular carcinoma in the 40–74 age group is 21.6% among males and 8.7% among females [5].

HCV is a small enveloped virus with a positive-sense, single-stranded RNA genome that encodes a large polyprotein consisting of three structural proteins and seven nonstructural proteins. The structural proteins of enveloped glycoproteins E1 and E2 are responsible for virus binding to the receptor molecules on cell surface, such as scavenger receptor class B type I (SR-BI), CD81, claudin 1, and occludin [1]. Meanwhile, the nonstructural proteins of p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B are responsible for viral RNA replication and viral particle construction [6–8].

The current treatment of HCV infection has markedly changed in the recent years. The direct acting antiviral agents (DAAs) combined with Interferon (IFN) have been approved and new IFN-free regimen combinations are recently available in many countries. DAAs targets nonstructural proteins of HCV resulted in the disruptions of the viral replication and infection. Currently approved DAAs consist of NS3 protease inhibitors such as simeprevir, asunaprevir and vaniprevir; NS5A inhibitors such as daclatasvir and ledipasvir; and NS5B RNA-dependent RNA polymerase (RdRp) inhibitors such as sofosbuvir [9,10]. The current treatment regimen using DAAs has dramatically improved sustained virological response (SVR) in most patients of different HCV genotypes. However, the emergence of drug resistance virus, safety for long usage, expensive cost of DAAs therapy, and limited access to the treatment, especially for patients in countries with relatively low income remain major barriers to HCV treatment. Thus, development of effective and inexpensive anti-HCV agents is still required.

Tropical rainforests exhibit a vast diversity in plants and those plants are sources for potential drug development. It has been previously reported that anti-HCV activities of Indonesian medicinal plants, in which 4 out of 21 plants extracts revealed anti-HCV activity against the HCV J6/JFH1-P47. One of the four plants was *Ficus fistulosa* known as Moraceae family [11]. Moraceae family consists of 60 genera and includes 1 400 species. The important genus of the Moraceae family is *Artocarpus* which is composed of 50 species [12]. *Artocarpus* is known to have wide bioactivities against virus [13,14], bacterial [15,16], malarial [17,18], and fungi [19,20].

In this study, three species of *Artocarpus* from Purwodadi Botanical Gardens, East Java, Indonesia, namely *Artocarpus altilis* (*A. altilis*) (breadfruit), *Artocarpus camansi* (*A. camansi*) (breadnut), and *Artocarpus heterophyllus* (*A. heterophyllus*) (jackfruit) were screened for anti-HCV activities.

2. Materials and methods

2.1. Cells and viruses

A clone of human hepatocellular carcinoma-derived Huh7 cells, Huh7it-1 [21], was cultivated in Dulbeco's Modified Eagle Medium (GIBCO Invitrogen, Carlsbad, CS, USA) supplemented with 10% Fetal Bovine Serum (Biowest, Nualle, France), 0.15 mg/mL Kanamycin (Sigma–Aldrich, St. Louis, MO, USA) and non-essential amino acids (GIBCO-Invitrogen) in 5% CO_2 at 37 °C. A cell culture-adapted HCV variant was propagated as described previously [9]. In brief, Huh7it-1 cells (1.8×10^7 cells) were infected with JFH1 1.8×10^7 focus-forming unit (ffu) for 4 h with agitation in every 30 min. The HCV-infected cells were incubated for 5 d. The supernatants at day 3 post-infection were collected and used for antiviral experiments.

2.2. Preparation of crude extracts

The leaves of *A. altilis*, *A. camansi*, and *A. heterophyllus* were obtained from Purwodadi Botanical Garden, Indonesia and verified by a licensed botanist. These *Artocarpus* leaves were extracted with several solvents (ethanol 80%, hexane, dichloromethane and methanol). The leaves were extracted using *n*-hexane and ethanol 80%. Meanwhile, the residue from *n*-hexane extract was further extracted using dichloromethane (DCM). Thereafter, the residue from dichloromethane was extracted using methanol. All extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and then stored at −30 °C.

2.3. Virus titration and immunostaining

Virus titration and immunostaining was performed as described previously [9,21,22]. In brief, virus supernatants diluted in the medium and inoculated onto the Huh7it-1 cells. After virus absorption for 4 h, the cells were cultured with medium containing 0.4% methyl cellulose (Sigma–Aldrich) for 41 h. The cells were fixed with 10% formaldehyde solution and permeabilized with 0.5% triton X-100 in PBS. The cells were stained with anti-HCV patient anti-serum and HRP-goat anti-human Ig antibody (MBL). The HCV antigen positive cells were visualized with Metal Enhanced DAB substrate kits (Thermo Fisher Scientific, Rockford, USA) and infectious foci were counted under microscope.

2.4. Antiviral activity assay

Antiviral activity assay was performed as described previously [9,21,22]. Huh7it-1 cells (5.2×10^4) were inoculated with HCV at multiplication of infection (MOI) of 0.1 in the presence of different concentrations of plant extracts (100, 50, 25, 12.5, 6.3 and 3.1 μg/mL). After virus absorption for 2 h, the cells were rinsed with the medium and were further incubated in the medium containing the same extracts for 46 h. For time-of-addition experiments, the cells were treated with the medium containing extracts only during viral inoculation (entry event) or only after viral inoculation (post entry event). Culture supernatants at 48 h post-infection were collected for virus titration. The 50% inhibitory effect (IC_{50}) was calculated by SPSS probit analysis.

2.5. Virucidal activity assay

Virucidal activity test was performed as described previously [9]. In brief, the HCV suspension (10^6 ffu/mL, 75 μL) was mixed with an equal volume of DCM extract and incubated for 2 h at 37 °C. Following by inoculated the virus suspension to the cells and incubated for 4 h at 37 °C. After removing viral inoculum, the cells were overlaid with 0.5% methyl cellulose-containing medium and incubated for 41 h.

2.6. Effect of host expression assay

The extract of DCM from *A. heterophyllus* was preincubated with cell (5.2×10^4) for 2 h at 37 °C. Then, cells were inoculated with HCV (MOI of 0.1) for 4 h. After viral absorption, cells were replaced with medium and incubated for 41 h. The culture supernatant was collected for virus titration and immunostaining.

2.7. Immunoblotting

The cells were lysed in a sodium dodecyl sulfate (SDS) sample buffer and the protein concentrations were determined using a bicinchoninic protein assay kit (Thermo Fisher Scientific). Equal amounts of proteins were separated in SDS-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane (Millipore, Bed-ford, MA, USA). The membranes were probed with an HCV NS3 mouse monoclonal antibody (clone H23; Abcam, Cambridge, MA, USA), an HCV NS5A mouse monoclonal antibody (clone 7B5; Biofront, Tallahassee, FL) or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (MBL, Nagoya, Japan) as primary antibodies followed by HRP-conjugated goat anti-mouse immunoglobulin (MBL) as the secondary antibody. Target proteins were visualized using enhanced chemiluminescence detection system (Biorad; GE healthcare, UK).

2.8. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

RNA extraction, cDNA synthesis, and qRT-PCR were performed as described previously [9]. In brief, RNA was extracted from cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). One μg of total RNA was transcribed using a ReverTra Ace qPCR RT Kit (Toyobo, Osaka, Japan) with random primers, and cDNA was amplified for real-time quantitative PCR using SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) in a MicroAmp 96-well plate. PCR was performed using ABI 7300 Real-Time PCR system with specific primers to amplify an NS3 region of the HCV genome 5′-CTTTGACTCCGTGATCGACT-3′ (sense) and 5′-CCCTGTCTTCCTCTACCTG-3′ (antisense).

2.9. MTT assay

The cytotoxicity of the samples was assessed by 3-(4,5-Dimethylthiazol-2-YI)-2,5-Diphenyltetrazolium Bromide (MTT) assay as described previously [9]. In brief, cells in 96 well plates were treated with various concentrations of crude extracts for 48 h. The medium was replaced with MTT containing medium and incubated for 4 h. Insoluble precipitates were dissolved with DMSO and the absorbance at 560 nm was measured using a microplate reader. The percentages of cell viability were compared to the control and calculated for 50% cytotoxic concentration (CC_{50}) values.

2.10. Data analysis

Results were expressed as mean ± SD. Differences between two data sets were evaluated by Student's two-tailed *t*-test. A *P*-value of < 0.05 was considered as statistically significant.

3. Results

3.1. Anti-HCV activities of A. altilis, A. heterophyllus, and A. camansi

We prepared crude extract samples from three *Artocarpus* species (*A. altilis*, *A. heterophyllus*, and *A. camansi*) using four various solvents (80% ethanol, *n*-hexane, DCM, and methanol) and obtained a total of 12 samples. Those samples were used for antiviral screening against HCV (JFH1 strain). HCV was inoculated onto Huh7it-1 cells in the presence of the samples and after viral adsorption for 2 h, the cells were extensively rinsed and further incubated in the same extracts containing medium for 46 h. The 50% HCV inhibition concentration (IC_{50}) and the 50% cytotoxic concentration (CC_{50}) and selectivity indexes (SI: CC_{50}/IC_{50}) of tested samples are shown in Table 1. The results showed that 6 of the 12 samples possessed strong anti-HCV activities with IC_{50} value of < 10 μg/mL and 3 extracts moderate activities with IC_{50} value of (10–20) μg/mL. Among samples possessing anti-HCV activity, the DCM extract of *A. heterophyllus* exhibited the strongest activity with an IC_{50} value of 1.5 μg/mL and $CC_{50} > 200$ μg/mL (SI: > 134.8). Methanol extract of *A. heterophyllus* and 80% ethanol extract of *A. heterophyllus* showed anti-HCV activities with IC_{50} values of 6.8 μg/mL and 12.9 μg/mL, respectively without any cytotoxicity effect with CC_{50} values > 600 μg/mL and > 800 μg/mL and SI values of 88.6 and 62.1, respectively. The DCM extracts of *A. altilis* and *A. camansi* revealed stronger anti-HCV activity with IC_{50} values of 6.5 μg/mL and 9.7 μg/mL, respectively, with CC_{50} value of > 50 μg/mL. On the other hand, the hexane extracts of the three *Artocarpus* species did not exhibit significant anti-HCV activities at the concentration of 100 μg/mL. Dose-dependent inhibition of HCV infection and cell viability of each sample was shown in Figure 1.

Table 1
Anti-HCV activity (IC_{50}) and cytotoxicity (CC_{50}) of *A. altilis*, *A. heterophyllus* and *A. camansi*.

Sample		IC_{50} (μg/mL)	CC_{50} (μg/mL)	SI
A. altilis	80% Ethanol	8.9 ± 0.3	> 50	> 5.7
	Hexane	> 100	> 500	NA
	DCM	6.5 ± 0.3	> 50	> 7.8
	Methanol	10.7 ± 1.6	> 200	> 18.6
A. heterophyllus	80% Ethanol	12.9 ± 2.6	> 800	> 62.1
	Hexane	> 100	> 400	NA
	DCM	1.5 ± 0.6	> 200	> 134.8
	Methanol	6.8 ± 0.8	> 600	> 88.6
A. camansi	80% Ethanol	6.7 ± 0.9	> 50	> 7.5
	Hexane	> 100	> 500	NA
	DCM	9.7 ± 1.1	> 50	> 5.2
	Methanol	13.0 ± 0.7	> 100	> 7.8

Data represent mean ± SD of data from triplicate experiments. NA, not applicable, SI, selectivity index.

3.2. Mode of action of extracts of A. altilis, A. heterophyllus and A. camansi

To determine the inhibitory stage(s) of extracts possessing anti-HCV activities in HCV life cycle, we conducted time-of-addition experiments, in which three sets of experiments were done in parallel: First, the extract(s) and virus were co-added onto the cells for 2 h and after virus adsorption, the cells were further incubated in the presence of the same extracts for 46 h (treatment during entry and post-entry steps). Second, the extract(s) and virus were co-added onto the cells for 2 h and after virus adsorption, the cells were further incubated in the absence of the extracts for 46 h (treatment during entry step). Third, the HCV virus without sample was inoculated onto the culture cells. After virus adsorption for 2 h, the infected cells were incubated

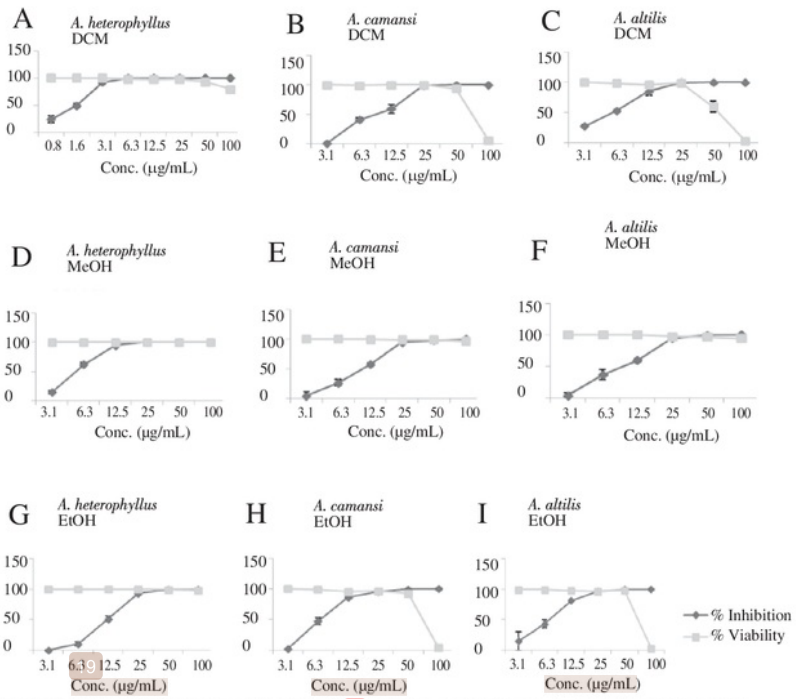

Figure 1. Dose-dependent inhibition of HCV infection and cell viability from *A. heterophyllus*, *A. camansi*, and *A. altilis*. Percent inhibition of HCV infection and cell viability by the extracts compared to the untreated control are shown. Data represent mean ± SD from three independent experiments. DCM: dichloromethane, MeOH: methanol EtOH: 80% ethanol.

with media containing same extracts for 46 h (treatment after virus entry). The results showed that all the three extracts (80% ethanol, DCM, methanol) of *A. altilis* and *A. camansi* exerted anti-HCV activity mainly at the post-entry event (Table 2). In contrast, the extracts of 80% ethanol, DCM and methanol of *A. heterophyllus* exhibited HCV inhibition principally at viral entry, while the post entry steps showed the lesser extent (Table 2).

3.3. Thin layer chromatography (TLC) analysis of extracts of A. altilis, A. heterophyllus, and A. camansi

Bioactivities of medicinal plants were influenced by the chemical contents of the plants.

Screenings of the bioactive components in the extracts were performed by TLC analysis and the result was demonstrated in Figure 2. The DCM, methanol and 80% ethanol extracts of *A. altilis* and *A. camansi* showed to contain with flavonoid component which is indicated by one major orange spot. On the other hand, TLC of methanol and DCM extracts of *A. heterophyllus* showed the presence of terpenoid and steroid which are indicated by purple and blue spots, respectively. DCM extract of *A. heterophyllus* also contained chlorophyll-related compounds with detection of red spots under detection of UV irradiation 365 nm. While the hexane extracts of *A. altilis*, *A. heterophyllus*, and *A. camansi* showed the presence of terpenoids as one major spots.

3.4. DCM extract of A. heterophyllus inhibits HCV infection through a direct virucidal effect and affecting host cells

Since the strong inhibition of DCM extract, further analysis to conduct the mechanism of action was performed for DCM

Table 2
Mode of action of crude extracts from *A. altilis*, *A. heterophyllus*, and *A. camansi*.

Sample	Solvent	Conc. (μg/mL)	% Inhibition			Mode of action
			During + post inoculation	During inoculation	Post inoculation	
A. altilis	80% Ethanol	30	97.5	3.1	98.1	Post-entry inhibition
	DCM	30	99.0	7.7	98.8	Post-entry inhibition
	Methanol	30	92.4	9.7	90.1	Post-entry inhibition
A. heterophyllus	80% Ethanol	25	89.4	56.7	15.0	entry inhibition
	DCM	6	91.0	81.1	22.5	entry inhibition
	Methanol	25	95.5	72.3	19.9	entry inhibition
A. camansi	80% Ethanol	30	97.1	10.6	93.9	Post-entry inhibition
	DCM	30	97.0	12.9	96.4	Post-entry inhibition
	Methanol	30	94.0	17.3	95.3	Post-entry inhibition

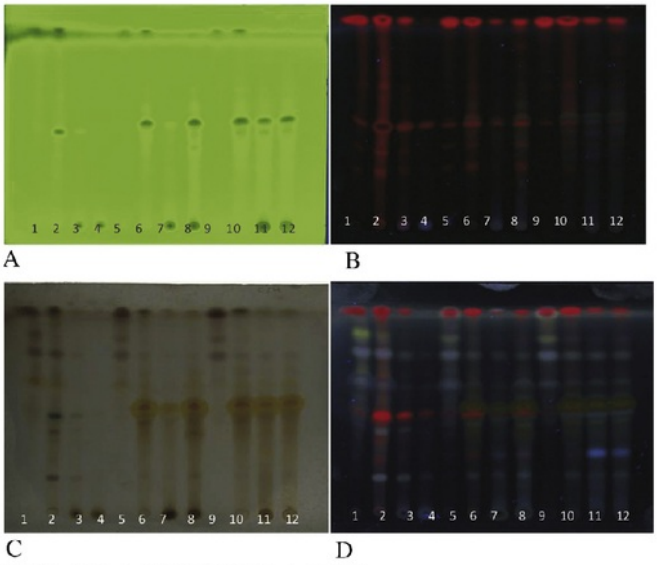

Figure 2. TLC analysis of extracts of *A. altilis*, *A. heterophyllus* and *A. camansi*.
Silica gel F254 TLC was used as stationary phase and chloroform:methanol (9:1, v/v) as mobile phase. Detection under (A) UV 254 nm, (B) UV 365 nm, (C) heating TLC plate at 105 °C for 5 min after spraying 10% sulfuric acid and (D) observed under UV 365 nm after using spray reagent and heated. Sample: (1) hexane extract of *A. heterophyllus*, (2) DCM extract of *A. heterophyllus*, (3) methanol extract of *A. heterophyllus*, (4) 80% ethanol extract of *A. heterophyllus*, (5) hexane extract of *A. altilis*, (6) DCM extract of *A. altilis*, (7) methanol extract of *A. altilis*, (8) 80% ethanol extract of *A. altilis*, (9) hexane extract of *A. camansi*, (10) DCM extract of *A. camansi*, (11) methanol extract of *A. camansi*, (12) 80% ethanol extract of *A. camansi*.

extract of *A. heterophyllus*. Time-of-addition experiment to determine the effect of extracts in the entry or post entry steps of HCV life cycle showed that the DCM extract of *A. heterophyllus* exerts anti-HCV activity at mainly in the entry-step. To evaluate whether the DCM extract acted on the target of HCV or host cells, the infectivity of the extract-treated HCV virions were determined. HCV virions were treated with the DCM extract or medium as the control for 2 h at 37 °C and then pretreated-HCV was inoculated onto Huh7it-1 cells for virus titration. As shown in Figure 3A, pretreatment of HCV inoculum with the DCM extract (6.3 μg/mL) was significantly decreased the HCV infectivity upon to 18.2% ($P < 0.00001$) compared with the untreated control (81.8% reduction). We next assessed the effect of DCM-pretreated host cells on HCV infection. Huh7it-1 cells were pretreated with the DCM extract for 2 h and then rinsed extensively to remove the extract. The pre-treated cells were challenged with HCV infection in the absence of the DCM extract. The result showed that the pretreatment of the cells with DCM extract (6.3 μg/mL) was significantly decreased HCV infectivity upon 9.9% ($P < 0.00001$) compared with the untreated control (90.1% reduction) (Figure 3B). These results suggested that the DCM extract of *A. heterophyllus* targeted both HCV virion and host cells. Since treatment of cells with the DCM extracts (6.3 μg/mL) revealed the effect at the post-viral adsorption step and also somehow inhibited HCV infection (Table 2), further confirmation of the effect on HCV RNA replication and HCV protein accumulation in the cells was evaluated. Real-time RT-PCR and immunoblotting analysis demonstrated that low concentration of DCM extract did not clearly supress HCV replication and HCV protein accumulation, however high concentrations of the DCM (> 12.5 μg/mL) acted to inhibit HCV replication (Figure 3C and D).

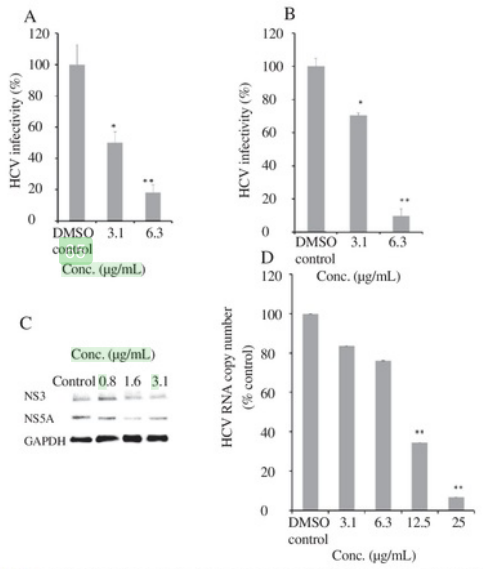

Figure 3. (A) Analysis of virucidal activity. HCV suspension was mixed with the DCM extract of *A. heterophyllus* for 2 h at 37 °C before inoculation onto the cells. (B) Effect of pretreatment of cells with DCM extracts of *A. heterophyllus* on HCV infection.
Cells were preincubated with the DCM extracts for 2 h and then challenged with HCV infection. (C) The HCV-infected cells were treated with the DCM extracts of *A. heterophyllus*. HCV protein accumulation in the cells was analyzed by western blotting against NS3, NS5A or GAPDH as a loading control. (D) The level of HCV RNA in the cells was measured by qRT-PCR. Data represent means from triplicate experiments ± SD. *$P < 0.001$ compared to the untreated control; **$P < 0.00001$ compared to the untreated control.

4. Discussion

Medicinal plants are potential resources for various bioactivities. Several components from medicinal plants have been reported to possess potential bioactivity including anti-HCV. Plants of *Artocarpus* genus have been used as traditional medicine in Indonesia for the treatment of fever, dysentery, and malaria. The genus of *Artocarpus* is rich in phenolic compounds, including flavonoid, stilbenoids, arylbenzofurans, and Jacalin (a lectin) [23–25] that were reported to possess a wild range of biological activities including anticancer, anti-inflammatory, antihypertensive, antibacterial, and antiviral [26].

In the present study, we screened crude extracts of three *Artocarpus* species: *A. altilis*, *A. camansi*, and *A. heterophyllus* for anti-HCV activities. A plant of *A. altilis* is known as breadfruit, which was used traditionally to treat liver disorders, hypertension, and diabetic. A total of 130 compounds were identified from *A. altilis*, of which more than 70 are derived from the prenylpropanoid pathways [25]. Meanwhile *A. camansi* is known with local name breadnut. The morphology of *A. altilis* and *A. camansi* is similar for leaves, fruits, and stems [25]. *A. camansi* is also believed to have similar medicinal properties to *A. altilis* [24].

Plant of *A. heterophyllus* has a local name Jackfruit and the leaves are usually entire (without lobes), much smaller than breadfruit and breadnut leaves. *A. heterophyllus* was known active as antibacterial activity against 24 species of bacteria [27]. Jacalin, a Jackfruit lectin from *A. heterophyllus* was reported to inhibit DNA viruses such as herpes simplex virus type II (HSV-2), varicella-zoster virus (VZV), and cytomegalovirus (CMV) [28], however, there is no reported yet about its anti-HCV activities.

Anti-HCV activities were demonstrated that 80% ethanol, DCM, and methanol extract of *Artocarpus* species mediated strong inhibition against HCV with IC_{50} value less than 15 g/mL, while the hexane extracts did not access any anti-HCV activities in the concentration of 100 g/mL (Table 1 and Figure 1). Further analysis was demonstrated that extracts of *A. altilis* and *A. camansi* exhibited HCV inhibition mainly at the post-entry step with percentage inhibition higher than 90%, while extracts of *A. heterophyllus* inhibit HCV in the entry step with percentage inhibition higher than 80% (Table 2). Common constituent(s) present in the extracts of *A. camansi* and *A. altilis* may exert similar anti-HCV activities.

To confirm the mechanism of *A. heterophyllus* how to inactivate the virus, virucidal activity and effect of host expression test were conducted. The result showed that pretreatment of the HCV virion or the Huh7it-1 cells with DCM extract of *A. heterophyllus* strongly reduced HCV infection. It suggested that DCM extract of *A. heterophyllus* exerts antiviral activities through direct virucidal activity and effecting host cells (Figure 2A and B) which may interfere the interaction with some receptors in the host cells. Some host cell molecules have been reported to be important entry factors or (co)receptors for HCV, such as glycosaminoglycan (GAG), low density lipoprotein receptor (LDLR), the scavenger receptor class B member I (SR-BI), the tetraspanin CD81, claudin-1 (CLDN1), and oclaudin (OCLN) which play as necessary keys in the attachments process of HCV to the host cells [29]. Since antiviral drug(s) targeting host factor(s) is generally known to lower emergence rate of drug resistance compared to the direct-acting antiviral drugs, the DCM extract of *A. heterophyllus* may be useful as a new drug development for the treatment of HCV especially to prevent HCV grant reinfection following liver transplantation.

Further analysis to confirm the effect of DCM extract of *A. heterophyllus* in the post entry step of HCV life cycle, western blot analysis was performed to examine the expressions of NS3 and NS5A HCV protein levels which played the important role in the replication of HCV. The result demonstrated mild inhibition of NS3 and NS5A protein expression level in DCM extract of *A. heterophyllus*-treated cells. Consistently, the HCV RNA levels were slightly inhibited by DCM extract of *A. heterophyllus*, however significant inhibition of RNA level was observed when the concentration was increased up to 12.5 and 25 μg/mL. These results were suggested the possible inhibition process attachment, assembly, release of virions and replication steps.

The bioactivities of components were influenced by the biochemical constituents among the plants. Our studies have not yet identified the compound(s) responsible for anti-HCV activity from *A. altilis*, *A. heterophyllus*, and *A. camansi* in this study. Other study was reported that *A. heterophyllus* contains lectin, artocarpine, artocarpetin, cycloheterophyllin, artonins A, morin, oxydihydroartocarpesin, cynomacurin, isoartocarpin, cyloartocarpin, artocarpesin, norartocarpetin, cycloartinone and artocarpanone. The leaves and stem are also reported to contain sapogenins, cycloartenone, cycloartenol, sitosterol and tannins [24].

Our TLC profiles were identified terpenoid and steroid components which served as major components of the DCM extracts of *A. heterophyllus* (Figure 2). The DCM, methanol and 80% ethanol extracts of *A. altilis* and *A. camansi* contained flavonoid as a major component. Some terpenoids have been reported to inhibit HCV infection such as Saikosaponin b2 from *Bupleurum kaoi* [30]; oleanic acid and ursolic acid [31]; Platycodin D, D2, D3, deapioplatycodin D, D2, platyconic acid A [32]; and andrographolide, a diterpenoid lactone from *Andrographis paniculata* [33]. Many flavonoids have also been reported to exert anti-HCV activity: Epigallocatechin-3-gallate (EGCG) [34], quercetin, luteolin, apigenin and ladanein [35], naringenin and silymarin/silibinin. The grapefruit flavonoid naringenin was reported to inhibit HCV assembly and release. Silibinin, the major component of silymarin, was reported to exert anti-HCV activity by blocking HCV entry, HCV fusion, replication and production of new progeny virus. These compounds are currently in phase 1 and phase 2/3 clinical trial studies, respectively [36,37]. As for the *A. altilis*, *A. heterophyllus*, and *A. camansi*, further analyses is required to determine the responsible compound(s) for anti-HCV activities in their extracts.

Extracts of *A. altilis*, *A. camansi*, and *A. heterophyllus* possess anti-HCV activity. The DCM extract of *A. heterophyllus* exhibits strong anti HCV activity through a direct virucidal activity and effecting host cells. The DCM extract of *A. heterophyllus* was a good candidate to develop a new antiviral agent to treat HCV infection and to prevent HCV grant reinfection following liver transplantation.

Conflict of interest statement

The authors declare no conflict of interests.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7